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Algae biodiesel: latest discoveries and market prospects

MEMÒRIA

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1 Summary

Pulsed Electric Fields is an innovative treatment based in the concept of electroporation (or electropermeabilitzation). This, despite not being fully understood, can be briefly described as the permeabilization of a cell membrane, result of the application of an electric field to the cell. This effect is used to break the cell walls that contain the lipids used in biodiesel production. The application of the electric fields in short pulses avoids undesired overheating problems as well as saves energy. Further understanding in the electroporation process will probably lead to better optimization of the PEF treatment.

PEF units basically consist of three units: High Voltage Pulse Generator, Treatment Chamber and Control System. The characteristics of the pulse generator and the treatment chamber such as pulse shape, resistivity and chamber geometry are determinant factors in the process. The system can be directly applied to the algae medium (wet biomass), without a typical drying step, this advantage dramatically reduces costs and energy consumption.

The process starts with the product (wet biomass) pumped to the treatment chamber there, it is under the effects of the pulsed electric fields generated with the pulse generator. This step, which only requires a few milliseconds to have effect is where the electroporation occurs. Hence the microalgae membranes are opened and the lipids released. Finally, a solvent such as chloroform, ethanol or hexane is used to recover the lipids from the medium.

This process permits an enhanced extraction of lipids and, at the same time, has many benefits for the overall process. Is easily scalable for industrial production, it can treat the full algae medium without a drying step, does not harm other valuable co-products derived from microalgae and reduces the use of solvents (usually hazardous and expensive).

2 Introduction

The increasing environmental problems, energy demand and limited fossil fuels as well as the energy independence motivation from many countries demands the search of viable alternative to the current fuel production. Biodiesels are a popular option due that they are relatively economic, environmental friendly and easy to implement in the current engines. Nevertheless, the growing world population will soon require most of the arable land for food production. Hence, traditional biomass feedstock from corps is incompatible with the upcoming world trends. Algae biodiesel however, may suppose a change. Algae do not compete with food production as it does not need arable land and, instead, can be cultivated in much small areas using special ponds. It can be cultivated in waste waters as source of nutrients and supplied with CO_2 to grow. It has shown much higher percentage of lipids production that any other feedstock (lipids are the material from which biodiesel is produced) and in less time as well as space. Although its known promising advantages, there are still many barriers that block its industrial commercialisation. One of these barriers is the lipid extraction, it is considered one of the bottlenecks of the system as it is very energy intensive and economically expensive between other inconveniences. The use of Pulsed Electric Fields (PEF) could solve this problem saving energy and costs to the process.

PEF is already used in genetics and more recently in food production. It is based in the phenomenon of electroporation, the permeabilization of a cell membrane under the application of pulsed electric fields. Despite of being reported a long time ago, it is a phenomenon still not fully understood. Its use in large industrial processes is still relatively new and many improvements and optimizations are yet to come. Nevertheless, it represents a promising future for algae industry, not only biodiesel, as it is economic, effective and does not require a high energy input.

This project aims to review the PEF technology pointed towards algae biodiesel production, it is structured in three points: a brief description of electroporation, the concept behind PEF; a description of the PEF system, its properties and factors; and finally, the implications in the algae biodiesel production.

3 Electroporation

3.1 Introduction to electroporation

"Superposition of an external electric field beyond a certain threshold value induces a transient and reversible permeability change of the membrane" (Zimmermann 1974)

A high intensity (kV/cm) electric field in the form of very short pulses (from µs to ms) applied to biological cells leads to the formation of pores in the cell membrane. This effect is called electroporation, it increases the overall permeability of the cell and, if maintained, causes the permanent breakdown of the membrane. This effect can be induced in microalgae to extract valuable products from within the cell such as lipids. The name of electroporation comes from its own nature (electric and poration), sometimes it is also called electropermeabilitzation. To study the process the cell membrane and its surroundings are considered to act as a capacitor. The membrane is a dielectric material that divides the external and internal conductive fluids.

Almost all eukaryotic cells (those from animals, plants and fungi), have a resting potential across the membrane due to different charges between the outside and the inside of the cell, this is more often called transmembrane potential. Usually, the positive charges remain outside and the negative inside. In an initial stationary state, this potential across the cell wall is of the order of 0,1mV (Coster and Zimmermann 1975).

When an electric field is applied, ions outside and inside the cell move and so the membrane gains a new potential. If this potential reaches the critical breakdown value, 1V for many cells (Sale 1976), it will lead to two different outcomes: reversible electrical breakdown, creating temporary pores in the membrane making it permeable; or irreversible electrical breakdown, causing the complete and permanent destruction of the membrane, cell lysis (Benz and Zimmermann 1979).

The breakdown and pore formation process present 3 different stages: first, pore opening and formation; after an increase of the size; finally, resealing if reversible or breakdown if irreversible. It is important to mention that while the process of forming

the pores is of the order of microseconds, the resealing takes around 4 seconds to complete (Chang 1992).

Moreover to the structural changes, there are also several changes in the characteristics of the membrane, such as an increase in the conductance of the membrane which leads to an increase in the current (in some points).

The complexity in the matter makes it difficult to predict reliable models as many factors can affect the result. The extracellular fluid surrounding the cell for example, can be composed of different substances making it a heterogeneous fluid with different electric and mechanic properties in a three-dimensional space. Moreover, processes like pore resealing are not fully understood. While in most theories the breakdown potential is given by certain, it is only an assumption for simplification as it is known since the first experiments that is in fact stochastic. The probability of rupture increases dramatically at the breakdown potential (Abiror 1979). Many theories try to describe the process, however some phases such as the resealing or the change of membrane conductance are not explained by many of these theories and further experimental proves are needed. As the process it is yet not fully understood, several models has been proposed. The electromechanical theory proposed by Crowley (1973) and Coster and Zimmermann (1975) considers membranes as viscoelastic objects. Applying electrostatics and elasticity concepts tries to define and predict the breakdown. The basis of theory is the compressive force produced by the increasing transmembrane potential. This compression decreases the membrane thickness what causes the membrane surface to increase and the lipid bilayer to destabilise. Below, a brief review of the basics of this theory are exposed.

3.2 Electromechanical instability theory

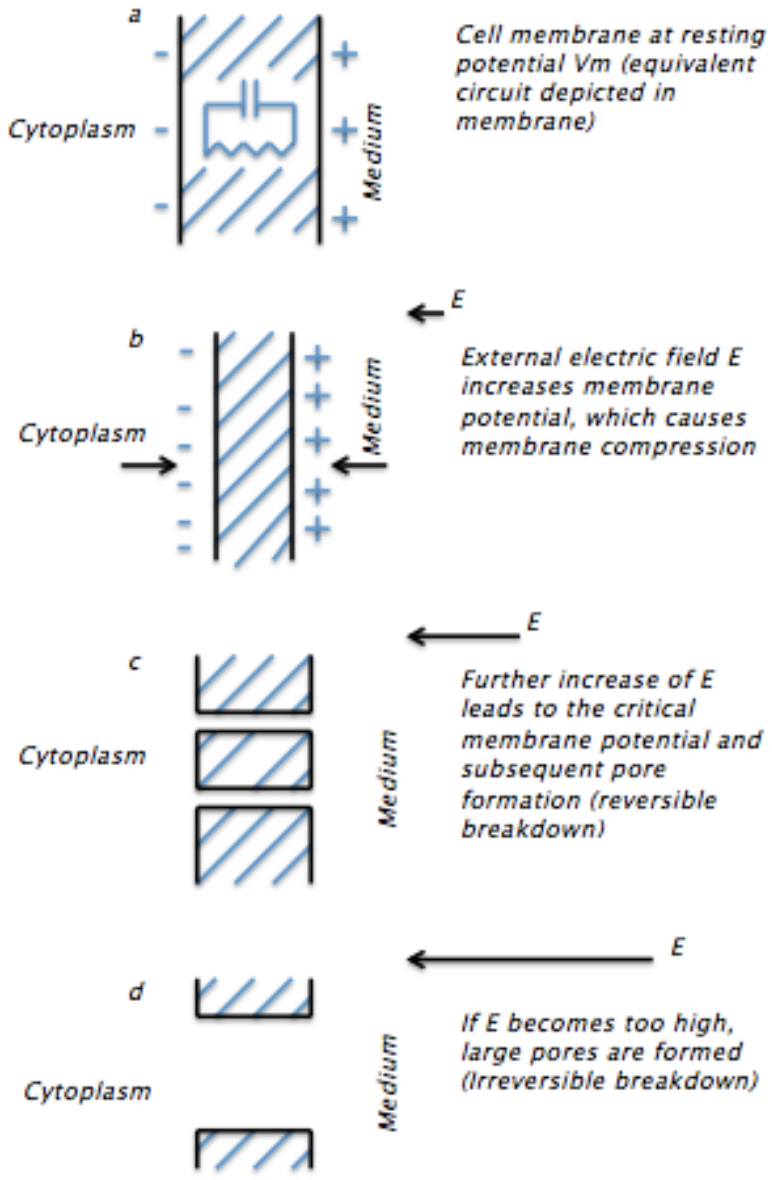

Figure 1 Cell membrane breakdown process. Adapted from Zimmermann (1986)

a) The membrane is considered as an elastic capacitor filled with dielectric. There are charges in both sides of the membrane that create a resting potential (about 0,1mV).

b) The application of an electric field E causes the ions to distribute along the field direction increasing the transmembrane potential, this potential is proportional to the electric field and the form (shape factor) of the cell. The increase of the transmembrane

potential produces an arising mechanical force that causes compression of the membrane.

c) The critical voltage Vc (1V) is reached, the mechanical force produces breakdown of the membranes and pores start to form perpendicular to the external electric field, where the highest potential is. Breakdown is reversible if the number and size of the pores are relatively small compared to the total surface of the membrane.

d) With higher field E intensity or longer exposure time, the pores become bigger and more frequent in the membrane. Irreversible breakdown occurs if this relation between number and size of pores is large compared to the total surface. This is the membrane destruction and the cell death.

As seen in Figure1, the electric field distributes the ions along the membrane surface. As a result, free charges accumulate on both sides of the membrane, what causes an increase of the transmembrane potential φ, higher than the resting potential. This new potential is distributed unevenly over the membrane as the ions move relatively to the direction of the electric field E (Figure2).

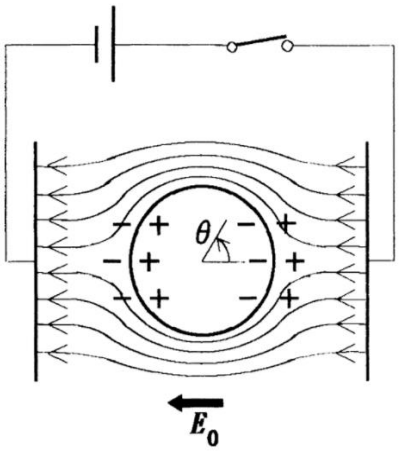

Figure 2 (Chang 1992) Representation of the free charges in a cell under an external electric field.

The pores start forming where higher compression forces are, these forces are caused by the higher potential, result of the ions distribution and finally the electric field. Due to this, the pores appear "perpendicular" to the external field E. The potential distribution before the appearance of pores is defined by (Eq.1):

$$\varphi(t,E,\vartheta) = -F\varepsilon_m E r_c \cos\vartheta\left[1-\exp^{-(\frac{t}{\tau_m})}\right] \qquad \text{Eq. 1}$$

Where φ is the transmembrane potential, F the shape factor, ε_m the relative permittivity of the membrane, E the applied electric field, r_c the cell radius, ϑ the angle (relative to the direction of the field and the point of study), t the time while the field is applied, τ_m relaxation time of the membrane (characteristic time constant). This equation (Eq.1) can be obtained from experiments as shown in (Figure3).

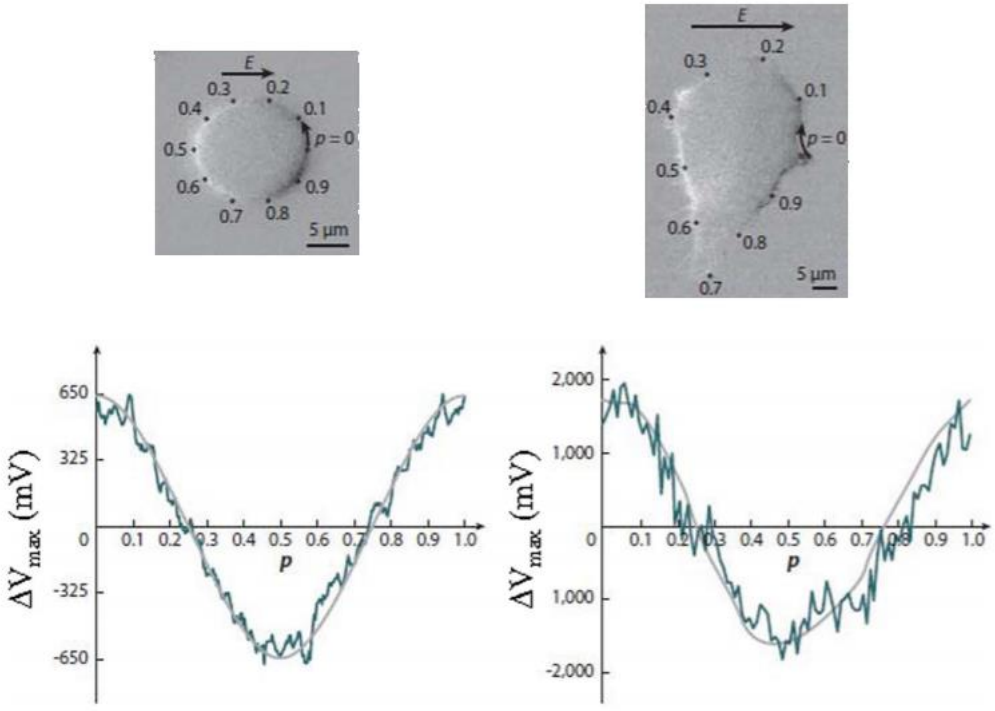

Figure 3 (Kotnik 2010) Two cells exposed to an external electric field. One has almost a spherical geometry (left) while the other is more irregular (right). Below the results of the transmembrane potential (TMP) for each point of the cell. It shows the influence of the direction of the field clearly followed by a cosine function around the cell.

The membrane permeability is obtained from (Eq.2):

$$\varepsilon_m = \frac{2\sigma_e\sigma_i}{(2\sigma_e+\sigma_m)(2\sigma_m+\sigma_i)+(\frac{r_c}{\delta})\sigma_m(2\sigma_e+\sigma_i)} \qquad \text{Eq.2}$$

Where σ_e, σ_i and σ_m are the electrical conductivities of the external medium, the cytoplasm (interior) and the cell membrane.

The equation (Eq. 1) is simplified when assuming that the membrane relaxing time is much larger than pulse duration (τ_m <<t). Those time independent function (Eq.3) is acquired:

$$\varphi = F\varepsilon_m E r_c \cos\vartheta \qquad \text{Eq. 3}$$

If the membrane is considered a pure dielectric (ε_m=1) and the cell, spherical, (F=1,5the equation (Eq.3) is reduced to (Eq.4):

$$\varphi = 1{,}5 E r_c \cos\vartheta \qquad \text{Eq. 4}$$

As seen above the highest potential occurs in the direction of the field E (if ϑ is the angle between the field and the point of study, this potential is find where ϑ=0). The highest potential is (Eq.5):

$$\varphi_{max} = 1{,}5 E r_c \qquad \text{Eq. 5}$$

Furthermore, the first pore will appear when this highest potential reaches the critical value (φ_c). Thus, the critical point is defined as (Eq.6):

$$\varphi_c = 1{,}5 E_c r_c \qquad \text{Eq. 6}$$

This equation (Eq.6) represents the critical potential to start pore formation and the critical field intensity E_c.

At the same time, the increase of the potential involves a proportional increase of the pressure supported by the membrane (Zimmermann 1976). Since the membrane volume is incompressible the area increases as the thickness decrease. This is, in fact,

the mechanical process that leads to the formation of pores. The pressure is defined by the following equation(Eq.7) (Crowley 1973):

$$P_e = -\frac{d}{d\delta}\int_0^{\delta}\frac{1}{2}\varepsilon_m\varepsilon_0 E^2 dx \qquad \text{Eq.7}$$

In this case P_e is the electro-compressive pressure, δ is the thickness of the membrane, E is the applied electric field and ε_0 the relative electric permeability of the empty space. Considering the compressive force independent from the position x, the equation (Eq.7) is simplified to the following (Eq.8):

(E== [V/m])

$$P_e = \frac{\varepsilon_m\varepsilon_0\varphi^2}{2\delta^2} \qquad \text{Eq.8}$$

On the other hand, this compression creates a strain force (a mechanical restoring force) of opposite effect across the membrane. Assuming now that it behaves as an ideal elastic material this force per area unit is (Eq.9):

$$P_m = Y\ln\frac{\delta}{\delta_0} \qquad \text{Eq.9}$$

Where Y is the elastic modulus of the membrane and δ_0 the initial thickness.

It is trivial then than for equilibrium, both forces (Eq.8 and Eq.9) are of equal modulus and opposite sign ($P_e+P_m=0$). (Eq.10)

$$\frac{\varepsilon_m\varepsilon_0\varphi^2}{2\delta^2} = -Y\ln\frac{\delta}{\delta_0} \qquad \text{Eq. 10}$$

When this equality (Eq.10) is not accomplished and the electro-compressive force exceeds the mechanical elastic strength breakdown occurs. Notice that for decreasing small values of δ (lim δ->0), P_e increases faster than P_m. The critical potential for breakdown is then (Eq.11):

$$\varphi_c = \frac{0.3679 Y\delta_0^{\,2}}{\varepsilon_m\varepsilon_0} \qquad \text{Eq. 11}$$

This expression proves that the breakdown potential is only dependent to the membrane characteristic.

Although these formulas may help to get a simplified idea, the exact mechanism of how the electric field interact with the membrane and pores remains unknown (Ho and Mittal 1996). There are several theories that try to describe the process but, without solid measurements on pore dynamics, there are yet no conclusions. Many of these theories try to explain the evolution of the lipid bilayer throughout the pore formation (Ho and Mittal 1996).

3.2.1 Molecular Reorientation Theory

A second theory which seems to have more supporters than the rest is the molecular reorientation theory, this considers as the main cause of the pore formation the effects and characteristics of the lipid bilayer and studies the matter with a molecular approach. A lipid bilayer is a polar membrane consists in two layers of lipid molecules. These membranes are flat sheets that form a wall around the cell and this is, indeed, the membrane of the cell. The lipid molecules consist of a hydrophilic head and a hydrophobic tail (See Figure 4 below). Hydrophobic is a molecule which is physically repealed from water, hydrophobic molecules are not soluble to water. On the other hand, hydrophilic molecules are physically attracted by water, soluble. With this, the cell membrane works as a barrier that keeps ions and other molecules from diffusing.

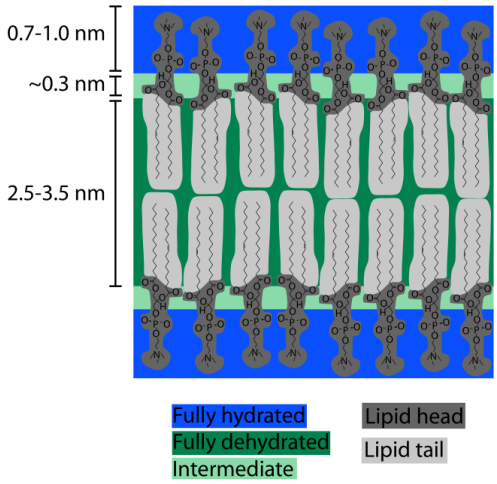

Figure 4 Representation of a lipid bilayer with its hydraulic phases. (Mashaghi 2012).

Neumann (1999) proposed a model to describe the process that ends in the pore formation that consists of two main steps (Figure 5). It begins with the elongation of the cell in the direction of the electric field that results with an increased curvature radius of the areas parallel to the field. This causes a slight increase in the membrane surface (around the 0.02%) while the volume remains constant. Under the action of both effects the density of the packaging decreases on the edges of the ellipsoid creating defects on the bilayer.

The second phase consist in the increase of the transmembrane potential. Discharge of this potential is believed to create conical hydrophobic pores. This augment the permeability and permit the pass of some ions. Under the continuous exposure to the electric field this early stage pores can evolve to a rearrangement of the bilayer into inverted hydrophilic pores, which then permit the pass of large hydrated molecules. If the electric field is not maintained, and after a certain time, the pores close themselves and the whole cell readjusts to its initial state. Otherwise the pores enlarge until the complete structure destabilizes and collapses, irreversible breakdown.

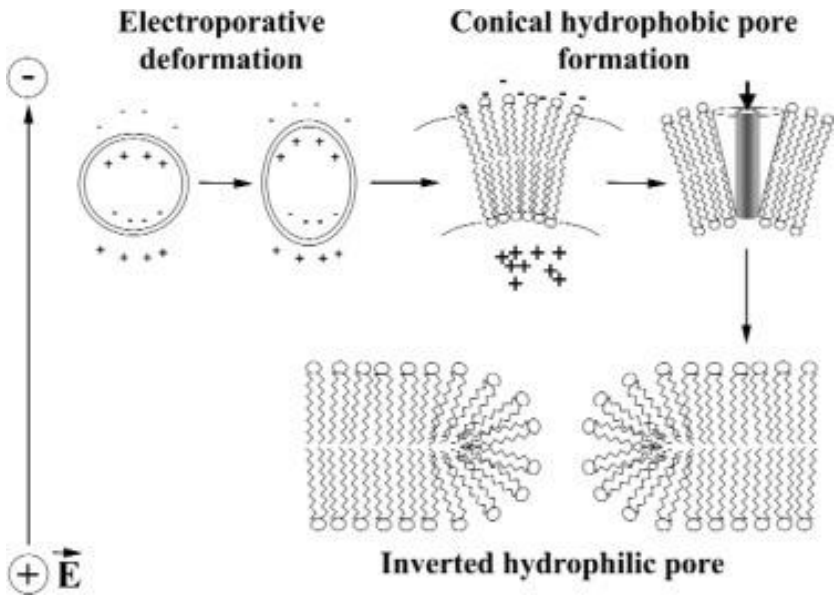

Figure 5 Pore formation process in the lipid bilayer. (Somiari 2000)

4 Pulsed Electric Fields

4.1 Introduction to Pulsed Electric Fields

Pulsed Electric Fields (PEF) treatment is a non-thermal and non-chemical technology widely used in the food industry as a process of preservation or extraction. It is based in the use of short electric pulses of (10 to 80 kV/cm) to induce electroporation to organic cells. Its effects can inactivate the bacteria or open the cell membrane without side effects on the food quality attributes. It is considered as a prominent technology for the biodiesel industry due to the energy efficiency, potential for scale up and economic in operations cost, compared to the other conventional methods.

The product is pumped through the treatment chamber, either in a continuous or a static design, where the product flows between a set of electrodes that confine the treatment gap. The generated voltage is supplied to the electrodes which then conduct it to the product placed in between. A cooling system may be used to help control the temperature created by the ohmic heating (See Figure 6).

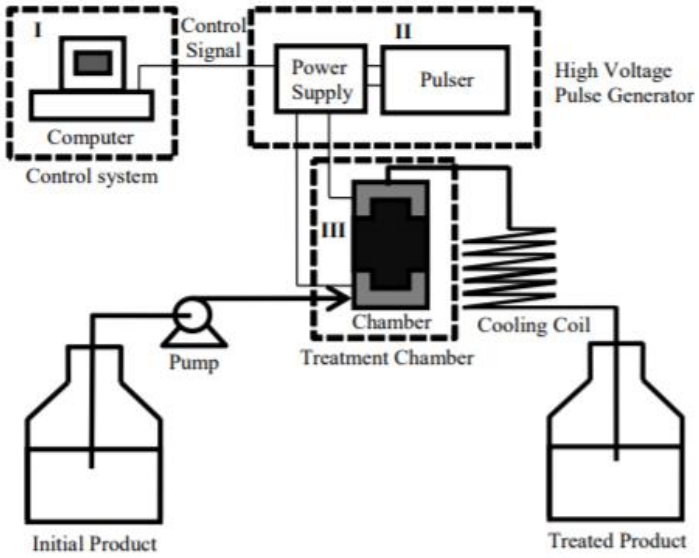

Figure 6 Schematic PEF system

The electric field can be applied in the form of exponential, decaying, square, bipolar or oscillatory pulse and at ambient temperature. The product conducts the electricity thanks to the presence of ions, which give a certain degree of conductivity. Depending on the intensity of the field the electroporation can be either reversible or irreversible. The electroporation process consists on the formation of pores and the partial or complete destruction of the cell membrane.

PEF systems are generally composed of three main elements:

(I) Control System to monitor the parameters

(II) High Voltage Pulse Generator that supplies the voltage

(III) Treatment Chamber where the product is processed

The process requires a fast discharge of electrical energy in a short period of time. This is accomplished by using a pulse forming network (PFN). More in detail, this is an electric circuit that consist of three main elements: one or more power supplies that provide the power, a pulse modulator that stores and discharges the high pulsed voltage and the treatment chamber. This pulse generator is also formed by several components that may slightly differ from one system to another, basically those are: a bank of capacitors, switches, resistors and inductors.

4.2 High Voltage Pulse Generator

The high voltage pulse generator provides electrical pulses of the desired intensity, duration and shape.

The power supply transforms the AC (alternating current) from the utility line into high voltage AC by a transformed and then rectified to high voltage DC (direct current). Then this power is used to charge the capacitor bank to the desired voltage. This energy remains temporally stored in the capacitors and then discharged very quickly, in form of pulse, to the product. This last discharge process is controlled by the switch.

The total power of the system is limited by the rate of the number of times the capacitor can be charged and discharged in a certain interval of time. The capacity of the pulse generator is usually expressed by the following relation:

Peak Power (MW) x Pulse Frequency (Hz) x Pulse width (μs) < Average power (of the power supply).

The power required to charge the capacitor, on the other hand, is defined by the resistors and the capacitance of the bank itself. As a review, the energy stored in a capacitor is given by (Eq.12):

$$Wc = \frac{1}{2} C\ U^2 \qquad \text{Eq.12}$$

From this it results more obvious that the more the capacitance, the more the energy that can store the bank.

4.2.1 SWITCHES

The discharge is accomplished with the use of a switch controlled by a high voltage trigger signal. Its characteristics define how much current and voltage can withstand and how fast can it work, because of these switches are one of the most sensible parts of the system. The most commonly used are: ignitrons, spark gaps, trigatrons, thyratrons and solid-state. Solid-state semiconductors such as IGBTs (Insulated Gate Bipolar Transistors) seem to be the best option for high power switches (Bartos, 2000).

Switches can be classified in two groups depending in its function: ON and ON/OFF switches. On switches can not be controlled to turn off, instead they turn off when the complete discharge is completed. Their operational margin relative to the voltage is higher than that from the other switches and with lower price. The major disadvantages of this devices are the low pulsing frequencies and short life span. Usually these devices are formed by a gas confined between two electrodes which is ionized to create conduction. As for the switches commented before the trigatron, ignitron, thyratron and spark gap work as ON switches.

On the other hand, ON/OFF switches permit a better control of the pulse generation with partial or complete discharge of the capacitors. This type of devices has been developed in recent years, mainly the solid-state switches. Especially semiconductor solid state switches present a much larger life span than the others, are easier to control, have better performance and have less energy loss. The drawback of this type of switches is that they can only withstand lower voltages, so it is necessary to use several units to increase its capacity. Thus, may result in a significant increase in the total price of the components.

4.2.2 PULSE WAVE SHAPES

4.2.2.1 Exponential decay

It is the simplest pulse, the voltage increases rapidly to a maximum and then decays slowly to zero. The simplest circuit model is a RC (resistance-capacitance) circuit as shown in the figure below (Figure 7). The circuit simply uses a switch to control the charges and discharges of the capacitor bank. When open, the capacitor stores the energy of the DC power supply. When the energy reaches a sufficient level the switches turns on, closing the circuit and enabling the discharge to the treatment chamber.

In fact, all the circuits used for PEF treatments reviewed in this paper are based on this system. With the addition of multiple switches and capacitors different kinds of wave shapes are achieved.

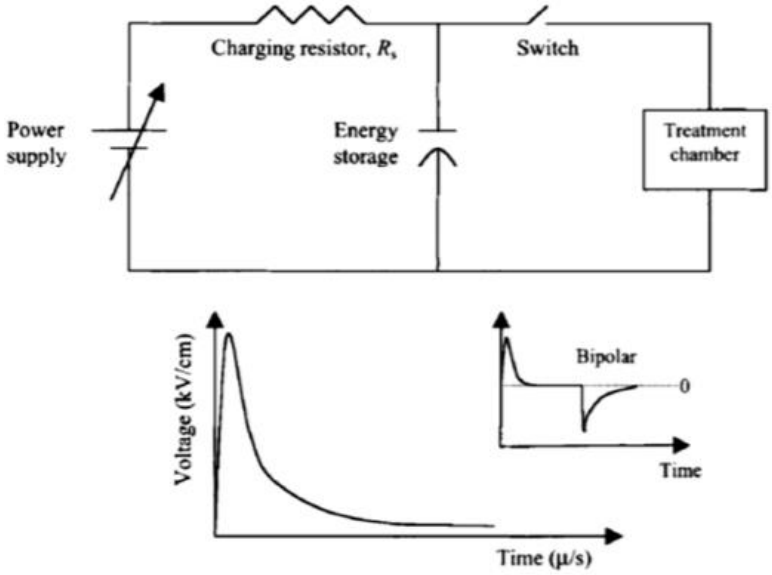

Figure 7 Simplified example of PFN circuit. The high voltage power supply charges the capacitor until a certain energy is stored and triggers the switch, what follows with the discharge process as a mono-polar exponential decay voltage across the treatment chamber. (Maged 2012)

The voltage across the treatment chamber is defined by the following equation (Eq.12):

$$V(t) = V_0 e^{-t/\tau} \qquad \text{Eq.12}$$

(Eq.12) corresponds to the graph of(Figure7), the voltage describes an exponential decay. In this case V_0 is the voltage charged in the capacitor, t the time of the pulse duration and τ (=RC) the time constant. R and C are the resistance and capacitance of the whole circuit; however, it can be assumed that $R=R_{Product}$ and $C=C_0$ as the resistance of the food is much bigger that thus from the electrodes and the circuit itself while the capacitance of the food is negligible compared to the one from the capacitors.

The pulse duration in the circuit above, is about five times the time constant (Cogdell, 1999). Nevertheless, most of the energy is discharged in a time equivalent to one time constant, when the voltage V(t) decreases to 37% V_0. Because this it is often assumed that all the energy is delivered in the first time constant. Many researchers use τ as the pulse width while some others still prefer using the total duration 5τ.

4.2.2.2 Square Pulses

It is also common the use of square shaped pulses, as the peak voltage is maintained constant during all the pulse duration (Figure 10). In theory, these pulses are generated by a transmission line, which implies two main problems. First, the difficulty to equal the impedance of the treated product with that from the transmission line itself. In practice, it is especially challenging to match those impedances for chambers with low resistance (<50 Ω). If this condition is not satisfied, the poorly matched impedances will degrade the signal and the shape will be distorted. However, if it is fulfilled, the pulse width is almost equal to the time constant (equal on ideal systems) which minimizes the energy loss by ohmic heating and simplifies assumptions and the extraction of conclusions. The second problem is that the transmission line it is not adequate for the pulse width used in PEF treatments.

These problems are both solved with the use of a pulse forming network (PFN) (Figure 9) as proposed in Zhang (1994a).

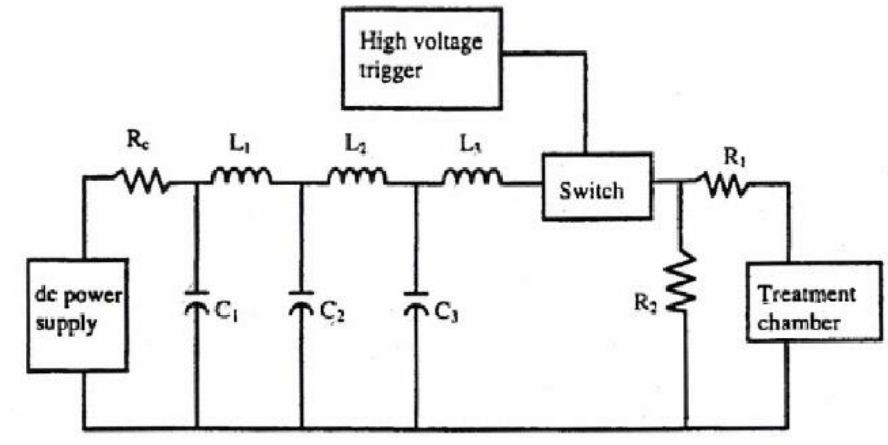

Figure 8. Representation of Pulse Forming Network (PFN) with three capacitor-inductor units used to generate square waved pulses (Zhang 1994a)

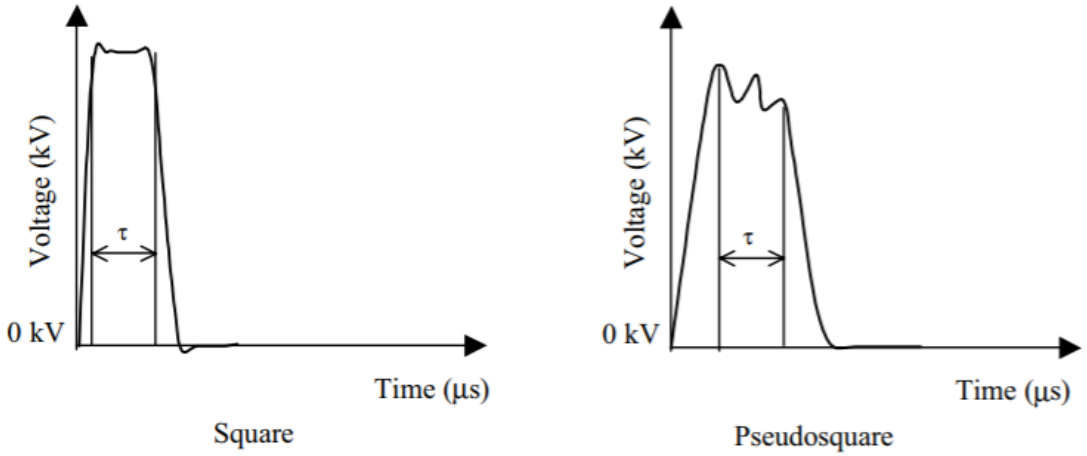

Figure 9. The first graph shows a square pulse with well-matched impedances while the second one is a pseudosquare pulse with a distortion (Góngora-Nieto 2002).

Exponential pulse generators are very simple and flexible while square pulses are more complex and expensive but also more efficient. (Zhang 1994a)

4.2.2.3 Bipolar Pulses

Bipolar pulses (Figure 12) can be generated via use of multiple switches. A practical example of a circuit is as follows (Figure 11):

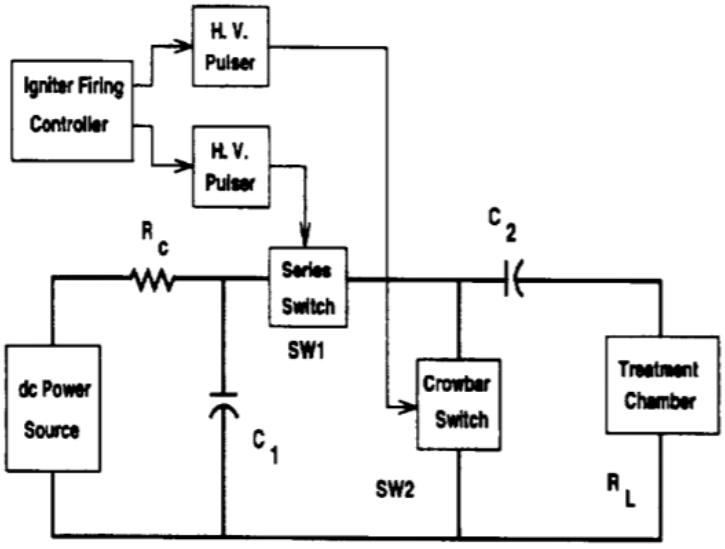

Figure 10. Circuit generator of a bipolar exponential decay pulse (Zhang 1994b)

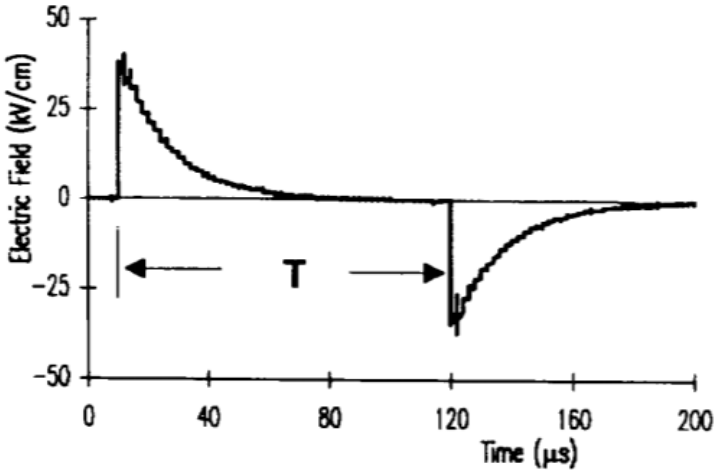

Figure 11. Waveform of a bipolar exponential decay pulse. (Zhang 1994b)

This circuit works with a pair of switches (one series and one parallel) and a pair of capacitors and resistors. The generation of the pulse works by combining the state of the switches, for a further understanding below are the different steps:

- Charging: When the series switch is SW1 is open the DC power supply charges the capacitor C1.

-First pulse (positive): SW1 closes enabling the pass of the current through the second capacitor C2 and the treatment chamber R_L. This process charges C2 at the same time that produces the positive discharge into the product.

-Second pulse (negative): As the first pulse voltage approaches zero SW2 closes and SW1 opens, this produces the second discharge, a negative pulse.

Repeating the process, the product is submitted to a series of bipolar pulses. A "complete" pulse is formed by a set of two discharges.

Even that some studies show that those are more efficient that monopolar pulses they are not as popular due to their higher cost (Zhang 1994b)

The lack of a consistent way of measuring the pulse width and the differences between ideal and real behaviour in the study of different pulse waves has become a limiting effect on the conclusions draw by the studies themselves, for that there is not yet a unique method for PEF treatment.

4.2.3 Pulse Energy Evaluation

The energy usage in a PEF system can be split in two: that used to run the system (power supply, switch trigger, computer, pumping, cooling...) and that delivered and used to directly treat the product. The electric pulse is the unique responsible to deliver the energy to the product, for this its shape and characteristics are critical aspects on energy efficiency (obviously, the energy related to the treatment).

Góngora-Nieto (2002) proposed using the following three basic parameters to measure the energy delivered to the product. These are function of the voltage and current across the product itself moreover to its characteristics.

The final energy received by the product in each pulse is (Eq.13):

$$En(t_1) = \int_0^{t_1} V_D(t) \cdot I(t) dt \qquad \text{Eq.13}$$

Where t_1 is the pulse duration $En(t_1)$ refers to the energy of a whole pulse), $V_D(t)$ and I(t) is the voltage and current across the product.

This first energy must be normalized for different sizes of treatments (Eq.14):. This is done using the energy density, defined as the amount of energy in a certain system or volume:

$$E_d = \frac{En(t_1) \cdot n}{V} \qquad \text{Eq.14}$$

Where E_d stands for energy density, *n* the number of pulses and V the volume of the treatment chamber with product affected by the pulses.

Even being a non-thermal process, the mere flow of current through the product (which acts as a resistor) produces ohmic heating. A cooling system is then used to reduce this undesired heating which involves an extra energy equal to that producing the heating. The temperature increase is defined as (Eq.15):

$$\Delta T = \frac{E}{\rho \cdot C_p} \qquad \text{Eq.15}$$

This equation is directly proportional to the energy and inversely to the characteristics of the product, ρ is the density of the product and C_p its specific heat.

For all this, not all the energy delivered to the product is entirely use it to produce electroporation. The main reason is that not all the intensities have an effect and otherwise, there is a minimum value for the electric field to have an effect. This implies that all the energy applied below the threshold is wasted (in ohmic heating for example). On the other hand, the field intensities above the limit are also relatively undesirable as the proportion of effect/energy is minor. The ideal pulse shape would be then, one that can "jump" directly to the threshold level and maintain the level until the end of the wave, a perfect square shape. With it most of the energy have a direct effect on the treatment.

On practice, a square wave has an energy efficiency of 91% whereas an exponential decay pulse, with the exactly same parameters (pulse duration, energy, energy peak...) has 64% (Zhang 1994b) (Figure 13). The results were exposed after an experiment with the objective to compare different waveforms for PEF treatment optimization. Whereas the exact value of the effectiveness may change under different conditions, the result clearly shows much higher efficiency in square pulses.

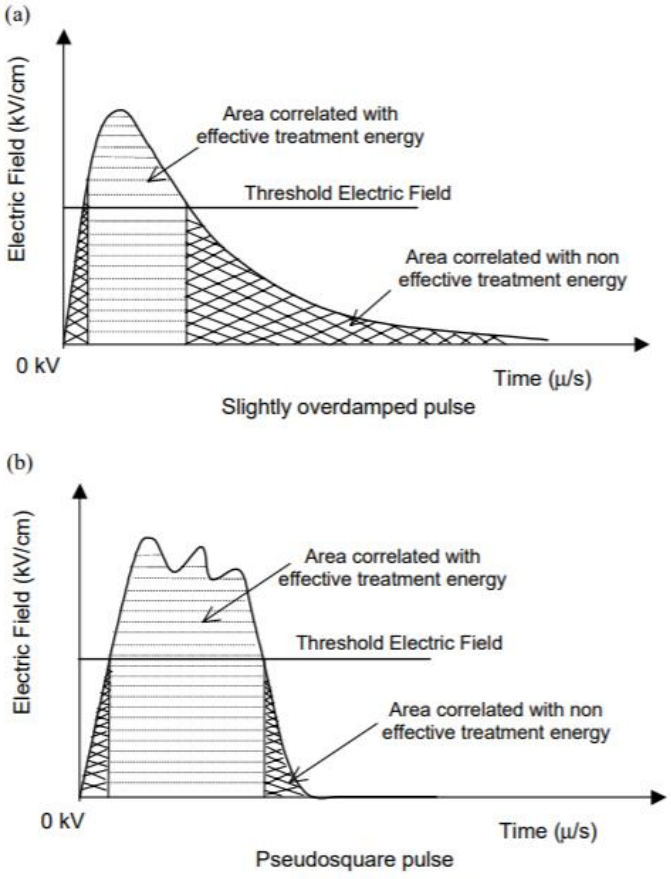

Figure 12 Comparison between the energy effectiveness of an exponential decaying (a) and a pseudosquare (b) pulse (Góngora-Nieto 2002)

On the other hand, monopolar and bipolar pulses also have different effects on the treatment performance. Although using the same amount of effective energy, the results presented in the same experiment, show that bipolar pulses have a higher level of inactivation that monopolar. It is believed to be a consequence of the structural fatigue (in the cell membrane) caused by the mechanical oscillation that polarity reverse causes (Chang 1989).

When treating liquids the effect of the electric field may produce electrolysis (chemical decomposition into smaller components, ions). Monopolar pulses usually create this undesirable effect leading to the separation of ions towards the respective electrodes, the accumulation of such charged particles causes a detrimental distortion of the electric field. Air bubbles created in the electrochemical process are also a matter of concern. In addition to perturbing the electric field, air under such conditions can suffer from dielectric breakdown what creates a flow of high intensity current through the air (Zhang 1995b). The current, that can damage the electrodes it is in fact, a point of concern in all kind of high voltage circuits as it is highly harmful for the electric components. The danger increases as treatment chambers are closed systems, in this case the breakdown not only affects the electrodes but also drastically increases the pressure inside the chamber which can lead to chamber explosions Zhang (1995b) and Zhang (2015). Bipolar waves nonetheless, change the electrodes polarity impeding the accumulation of and separation of ions.

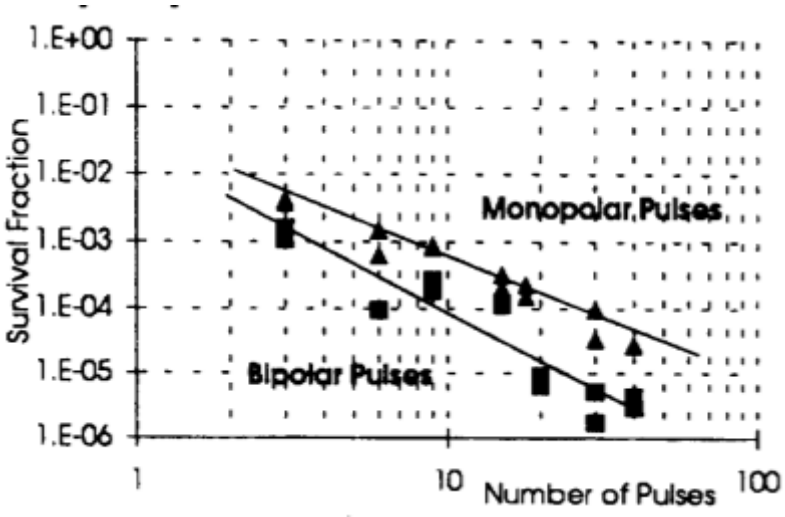

Figure 13. Comparison on the effects to the Survival Fraction of cells under monopolar and bipolar pulses (Zhang 1995b).

Square and bipolar pulses are more energy effective for PEF treatments (Figure 14), nevertheless differences in the circuit complexity also makes them more expensive that exponential decay and monopolar pulses.

4.3 Treatment Chamber

The second major component in PEF systems is the treatment chamber. Its objective is to transfer the electrical discharge from the electrodes to the treated product. It basically consists of two electrodes and an insulating material that houses the product and holds the electrodes. Many chamber designs have been developed and patented over the last 20 years Zhang (2015). since the U-shaped static treatment chamber designed in the 1967 by Sale and Hamilton. They can be classified into two groups: batch (static) and continuous. Static are more suitable for small scale laboratory studies while continuous chambers are better for prototypes and large scale industrial treatments. Regarding the electrodes geometry, parallel plates have been typically used for static treatments while coaxial and co-field for continuous flow, this last present higher inactivation rates due to better homogenization of the electric field influence in the circulating product (Qin 1998). (Figure 15)

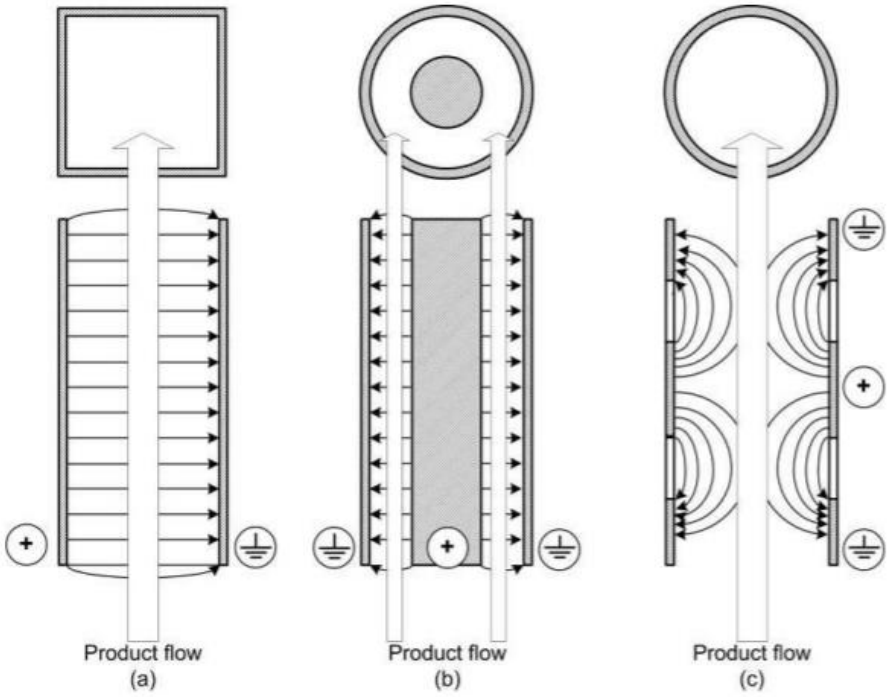

Figure 14. Simplified examples of three electrode configurations. From left to right parallel plates (a), two cylinders in coaxial disposition (b) and co-linear (c) (Barvosa-Cánovas 1999).

An adequate design of the treatment chamber is important for its proper functioning, many aspects regarding the geometry, size and materials of the electrodes and insulators are considered. In general, the key parameters of treatment chambers are: its ability to supply a uniform high electric field to all the product and the impact on the product flow (for continuous-flow chamber) (Salengke 2012). A good design is able to enhance the homogeneous distribution of the electric field while avoiding a reduction of the product flow. Systems with several continuous chambers connected are used in order to increase the product flow, they are widely used in industrial and large scale applications (Barbosa-Canovas 2002).

As the PEF process involves high voltage pulses it is important an adequate chose of the materials. The insulator must have a high dielectric strength able to withstand the strong electric field. Plenty of research has been made over the field of high voltage to parametrize the properties of a wide range of insulators. Some common used materials are: polythene, polypropylene, nylon, polysulfone, Plexiglas and PVC (Huang 2009). On the other hand, different materials for electrodes are still in research. This material needs to be conductive and able to withstand erosion, deposition and electrolysis. The first chambers used graphite because it was suitable for food processing, however, the electric current erode them rapidly resulting in short lifetime. Some of the materials currently used are: stainless steel, gold, platinum, titanium and metal oxides being the first the most common.

Other important parameters highly related with the system performance are: intrinsic electrical resistance and reduction of enhanced field zones (Barbosa-Canovas 2002). Zones like edges or vertexes in rectangular electrodes produce variations in the electric field. To avoid it, the electrodes usually have a circular like shape in order to use geometries that escape from vertexes.

The size and geometry of the design together with the conductivity of the treated product define the intrinsic electrical resistance of the chamber. This together with the resistance and capacitance of the other components composing the PFN determine the pulse width, the electric field peak and the energy density supplied. The intrinsic resistance of a chamber (R_{Ch}) is defined by (Eq.16):

$$R_{Ch} = \frac{d}{\sigma A} \qquad \text{Eq.16}$$

Where *d* is the distance between electrodes, σ is the electric conductivity of the product and A is the area of the electrodes.

To analyse it, the whole circuit is simplified as shown in the figure below (Figure 16).

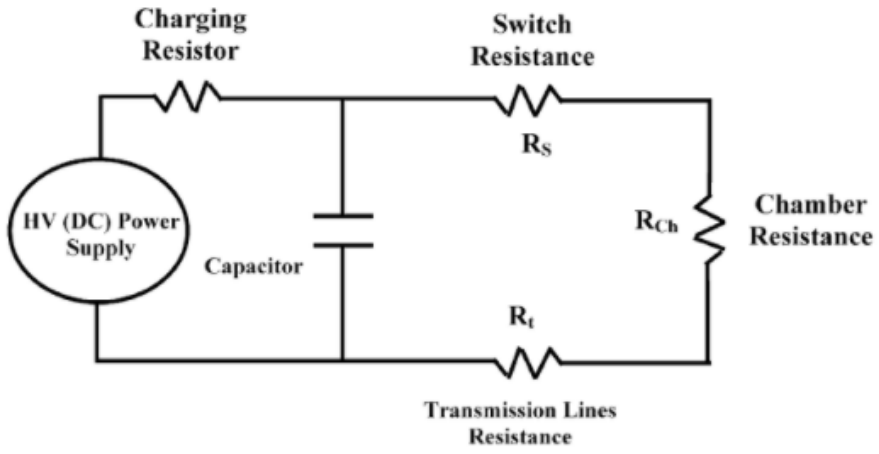

Figure 15 Representation of a simplified electric circuit for PEF treatment (Barbosa-Canovas 2002).

It is obvious that the total resistance (R_T) is equal to the sum of all resistances. The circuit acts as a voltage divider for what we can deduce that (Eq.17):

$$V_{Ch} = V \frac{R_{Ch}}{R_T} \qquad \text{Eq.17}$$

This implies that the resistance of the treatment chamber is directly proportional to its voltage. The more the chamber resistance, the more the voltage peak at the electrodes, and contrariwise. This important property is key for the system performance as it has direct influence on the voltage and electric field.

When the pulse comes from the discharge of a capacitor, the energy per pulse received by the product (En) is described as function (Eq.18) of the capacitor's energy and the ratio of RCh against RT (Barbosa-Canovas 2002):

$$E_n = \left(\frac{R_{Ch}}{R_T}\right) \cdot \frac{CV^2}{2} \qquad \text{Eq.18}$$

A line with high resistance in the chamber and relatively low resistance in the other circuit components ($R_{Ch}/R_T >>$) will have low loses in the circuit components, delivering most of the energy stored in the capacitor to the product. The high ratio of R_{Ch} to R_T creates high voltage levels on the chamber and low currents across it. This characteristic permits the use of lower capacitors and voltages, what generates low energy pulses compared with same treatments with low resistance chambers.

Furthermore, as the increase of temperature is highly related to the supplied energy, low energy pulses will produce less heating in the product. Following the equations defined in the past section, a more detailed energy balance can be obtained.

The number of pulses is defined by (Eq.19):

$$n = \frac{fv}{F} \qquad \text{Eq.19}$$

Where f is the pulse frequency, v the product volume (inside the chamber) and F the product flow. Then:

$$E = \frac{fvE_n}{F} \qquad \text{Eq.20}$$

Where E is the total energy supplied to the product. The energy balance for a continuous PEF treatment is then defined as (Zhang 2011):

$$\Delta T = \frac{fE_n}{\rho F C_p} \qquad \text{Eq.21}$$

It is now proved that most effective chambers are those with low energy input and low product heating, an optimised design will seek to increase the intrinsic resistance. As seen in a previous equation, resistance is proportional to electrodes distance and

inverse to product conductivity and electrodes area. If the product is defined, conductivity is fixed, for what only geometry variables govern the equation. Increasing the distance between the electrodes will increase resistance but, at the same time, decrease the electric field (Eq.22).

$$E = \frac{V}{d} \qquad \text{Eq.22}$$

Another option is to reduce the electrodes area, this on the other hand, has the drawback to reduce the volume of the chamber too.

In general, parallel and coaxial chambers have an intrinsic resistance of 3 to 30Ω while co-field designs have 50 to 300Ω (usually due to small electrodes surface and chamber volume) (Góngora-Nieto 2002).

Parallel plate electrodes permit a uniform distribution of the electric field nevertheless, some problems such as big electrode surface and difficulty for continuous flow treatment make it unsuitable for scale up process.

Coaxial chambers are better for continuous design whereas its drawbacks are the usually low intrinsic resistance and the radial distribution of the electric field (heterogeneous).

Co-field chambers have different densities of the electric field for each design as there is not a predefined configuration of the electrodes. Typically, they present high intrinsic resistance and better flow dynamics.

4.4 Control system

Electrical and process parameters are monitored by the control system. The computer controls the discharge through triggers and the charging periods. As for monitoring and data gathering, voltage sensors of high bandwidth and fast response are used to measure the input volts. An oscilloscope is used to determine the form and the value of the voltage. The electric field across the chamber is also controlled using specific equations for each treatment that relate the voltage and the electric field. Other data such as current flow or temperature are measured with different probes.

4.5 Primary Factors in PEF

The results of pulsed electric fields treatment depend on several key parameters of the process such as the product treated, the characteristics of the pulse, the design of the chamber and treatment temperature between others. They are usually split into three families: technological, biological and media factors.

In general, most factors are extrinsic for what they can be controlled and easily modified to adjust to the desired value. However, some are intrinsic and related between each other for what they can not be modified independently nor directly.

Thus, an optimized performance implies knowing the significant factors of the process and the effect and interactions between them. The outcome of all the factors together are described by different models of equations and relations that form the inactivation/PEF kinetics.

4.5.1 TECHNOLOGICAL

Technical factors are those related with the equipment and its components as well as the parameters those involve. They are usually extrinsic, easily defined for the process and the optimization.

4.5.1.1 Electric Field Strength

It is widely known that the electric field is the most important factor in PEF treatment. It has a direct impact on the result of the treatment and a minimum threshold level must be reached for the process to have any effect at all. This level is the minimum that will produce a transmembrane potential in the range of 1V which causes membrane disruption. Higher electric fields enhance the result, however an excess of electric field strength may cause the dielectric breakdown of the product itself, for this reason the electric field is typically close to the threshold value. Non uniform electric field across the product may produce zones with very high electric field or zones with very low electric field, this differences may cause a dielectric breakdown or untreated product volumes as well as high variance in the result.

The effectiveness of the electric field is influenced by both cell size and orientation (Heinz 2001). The smaller the cell the higher the necessary minimum electric field to have effect. Different shapes behave differently to the electric field for what it effectiveness can change depending on each case.

4.5.1.2 Treatment Time

It is considered as the most important parameter for electroporation just after the electric field intensity. The treatment time is the total effective time that the product is under an electric field, it can be obtained by multiplying the pulse width by the number of pulses. PEF treatment is applied in short pulses to avoid the undesirable effects consequence of the application of the electric field, basically heating and electrochemical reactions.

It has been proved a direct relation between treatment time and survival rate, the more the time the less the survival rate (Figure 17) (Hülsheger 1981). Periodic pulses are considered to enhance performance, it is supposed that the improvement is due to: repetitive stress across the membrane and proper orientation of defects in each pulse (Barbosa-Canovas 2004).

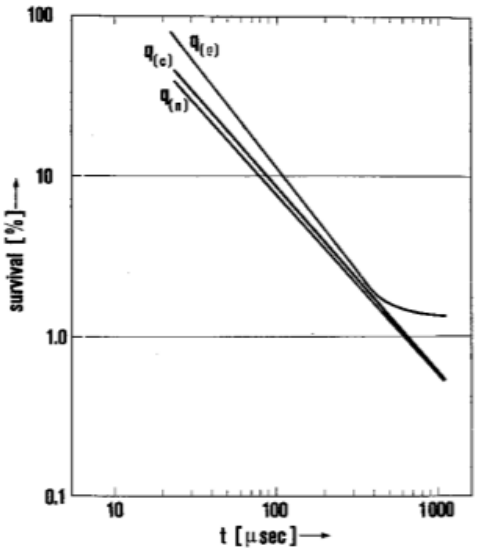

Figure 16 Graph representing the survival rate [%] as function of the treatment time $t=n\cdot\tau$ [μs] in a logarithmic scale. The relation is clearly logarithmic as it appears linear in the graph (Hülsheger 1981).

4.5.1.3 Energy Input

The applied energy is an intrinsic factor that depends from other factors such as voltage, treatment time and other pulse characteristics between others. Its effect on the result is not clear yet, probably due the strong relations to other factors. Even not having a direct impact in electroporation it may be true that the way the energy is applied and distributed between parameters (pulse shape, voltage, etcetera) may influence other factors, like product temperature, which would affect the result.

4.5.1.4 Pulse Shape

As seen in the previous sections different pulses result in different outcomes. The pulse shape is a critic parameter that must be well studied and defined in each treatment. It is also highly related to other factors, mostly this are the energy input, the electric field, treatment time and temperature. Modifying the wave form varies the results as well as the factors related to it, the effectiveness of the process closely dependent of it.

4.5.2 MEDIA

Media factors are those chemical and physical attributes usually inherent to the product. Most of these parameters are intrinsic to the product like pH or conductivity, and they can not be modified at will. The PEF treatment must be adapted to each product and its own characteristics. In general, extrinsic factors are adapted to media factors. They play an important role in the process as a good understanding of each factor influence, will allow better adjustment of the whole treatment towards further optimization.

4.5.2.1 Conductivity

As explained in past chapters conductivity is a key parameter in the PEF process (Figure 18). Conductivity is closely related to ionic strength, this is defined as the concentration of ions in a solution. Media rich in ionic molecules such as marine algae or tomato juice are harder to process under PEF as lower electric fields are reached due to lower voltage and higher current. It is known that conductivity can be modified by temperature

changes, high temperatures increase the mobility of ions trough the solution and at the same time the conductivity. Resistive heating may lead to conductivity increase which implies the intrinsic chamber resistance to decrease together with the electric field. To maintain the intensity of the electric field the voltage increases, this whole process implies a loss of effectiveness (Heinz 2001).

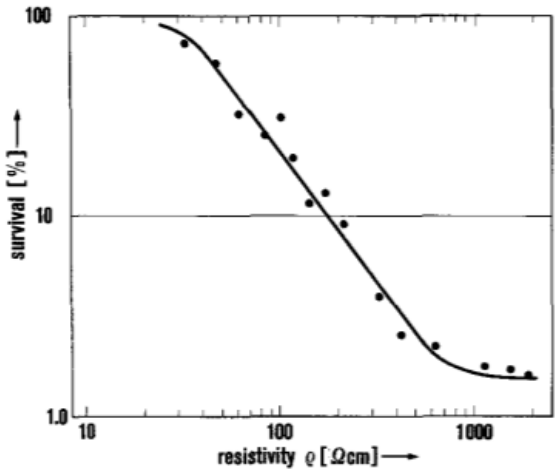

Figure 17 Survival rate as function of solution conductivity under the same PEF treatment conditions (Hülsheger 1981).

Although it is an intrinsic characteristic of the product its effects can be modified by the chamber geometry as mentioned in the past chapters. It is important to mention that while low ionic strength is optimum for the process, ions are the causing of the transmembrane potential and therefore needed in the process. Despite this, (Heinz 2001) proved that ion strength and hence conductivity, do not have a direct effect on transmembrane potential in pulses longer than 1μs.

4.5.2.2 Treatment Temperature

PEF is a non-thermal technology because the only cause of the process comes from an electric field, without the need of a certain temperature. Although it is not necessary for it, temperature does have an effect on the process (Figure 19). It has been reported in several studies over the years how higher temperature increases treatment efficiency (Zhang 2005). It is important to define that the temperature must be below a certain

value in order to distinguish between the effects of the temperature and the electric field, this level is usually around 65°C (338,15 K) (Barbosa-Canovas 2004).

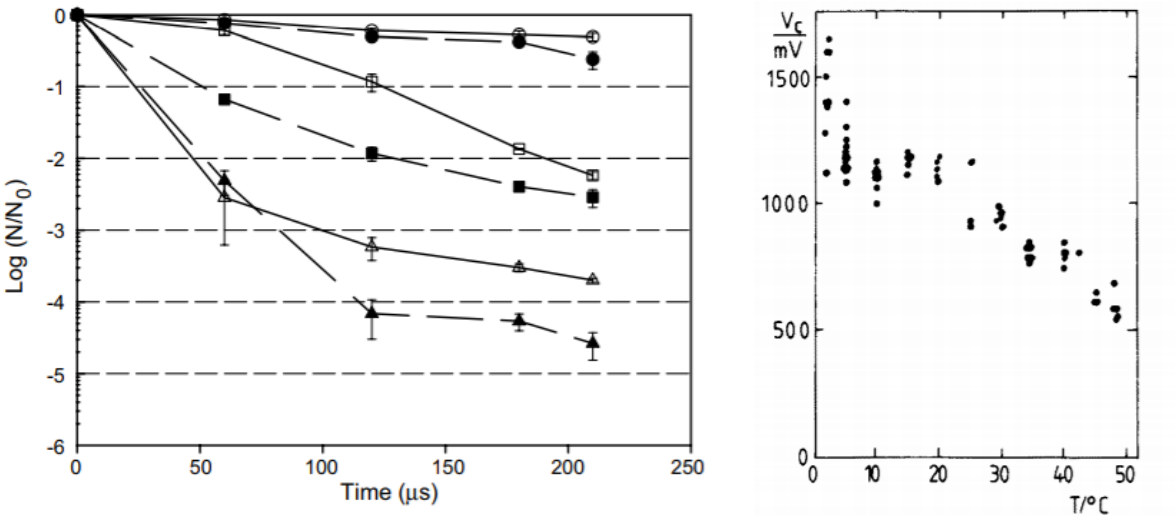

Figure 18 Two graphs showing the influence of temperature in the process (different bacteria where used). The first on the left shows the survival rate (logarithmic) function of treatment time (Time), temperature and electric field. Different characteristics are represented in three different forms: ○, □, Δ and two different fillings: empty (○) or full (●). Objects with the same filling have the same electric field: empty (○) E= 20kV/cm while full (●) E=30kV/cm. Objects with the same form have the same Temperature: ○ T=20 ℃, □ T=30 ℃, Δ T=40 ℃. It is seen how the temperature has a determinant effect on the results (Amiali 2007).

The graph on the right shows the relation between the critical voltage (Vc) to cause inactivation and the temperature (T). It shows a decreasing voltage as temperature arises.

It has been proposed that, the improve of performance at higher temperatures is due to a dependence of some membrane characteristics to the temperature. Thus, higher temperature will decrease the membrane mechanical resistance, for what a lower electric field will cause disruption. At low temperatures, the whole membrane behaves like a gel-like structure, while at rising temperatures it becomes more fluid also losing elasticity (mechanical resistance) (Beney 2001).

4.5.2.3 Dielectric Strength

The dielectric strength does not directly affect the process factors, nevertheless it is a limiting factor. As explained in past chapters, when the dielectric strength of a material is surpassed a potentially dangerous spark known as dielectric breakdown occurs, this may lead to the destruction of several system components. It is important to take into account the value of the dielectric strength of the product as well as to control the appearance of bubbles or other products that may alter it an affect the safety of the process.

4.5.2.4 Composition

The composition of the media such as the presence of proteins, substances or the pH level can also affect the result. It is important to adjust the treatment to each product, considering all these factors to obtain a uniform electric field and make the most of it.

4.5.2.5 Physical Attributes

Other physical attributes related to the treated media have an impact in the optimization of the process and are related to some other factors. Some of the most important are:

-Density and specific heat, the temperature increase, as seen before, has a direct relation with the energy received as well as with the density and specific heat.

-Thermal conductivity and diffusivity determine the heat flow distribution across the product.

-Viscosity and other extrinsic factors as velocity and chamber geometry determine the flow regime of the product (if pumped).

4.5.3 BIOLOGICAL

Particular characteristics of each microorganism are key factors for the process (Barbosa-Canovas 2004). Different species or even different cell shapes behave different under the same treatment conditions. Thus, factors are clearly intrinsic as they are

inherent to the objective cells. A proper PEF design requires a deep understanding of the microorganism characteristics to adjust the process to each case.

4.5.3.1 Size and Shape of cells

The "geometry" of the cell defines the electric field required to cause the critical transmembrane potential. As seen in past chapters, this potential is function of the shape factor. The cell shape factor (f) (Eq.22) is defined by the measures of the cell: the three semi-axes (A_1, A_2, A_3) and the distance A_F from the centre and in the direction of the electric field. Its function for elliptical cells: (Zimmerman 1974)

$$f = \frac{2}{2 - A_1 A_2 A_3 \int_0^{\infty} \frac{1}{((s + A_F^2)(\sum_{n=1}^{3} \sqrt{s + A_n^2}))} ds} \qquad \text{Eq.22}$$

In general, the smaller the cell the higher the electric field needed to induce the critical potential. Different cell shapes also imply different breakdown characteristics (Figure 20). Several researchers have studied the topic over the years since the first electroporation models (EL-Hag and Jayaram 2008):

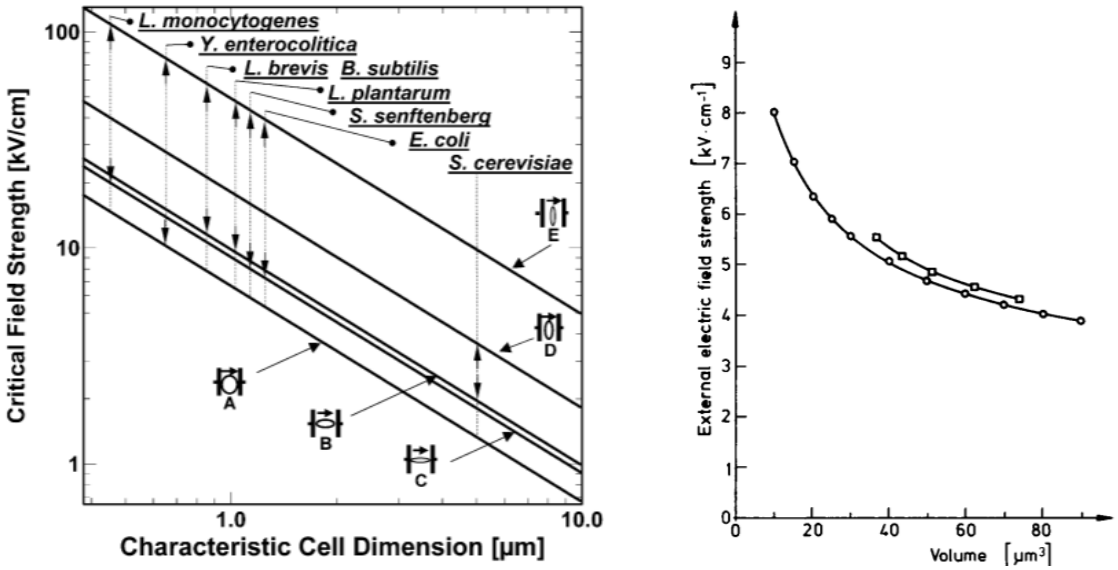

Figure 19 Two graphs showing the critical electric field as function of the cell factors shape (right image) and size (left image). (Zimmerman 1974) and (Heinz 2001)

4.5.3.2 Type of Microorganism

Each species has different results under the effect of pulsed electric fields. Whereas same species of either bacteria, yeast or algae behave between certain boundaries different strains may be particularly resistance despite the whole population average. For this, although it is positive to know the general behaviour of a certain specie it can only be used as a general idea as each strain may behave differently (Barbosa-Canovas 2004).

4.5.3.3 Growth Stage

It has been repeatedly reported that the growth stage of the cell media is a factor of importance for PEF treatment (Figure 21). In general cell populations have three differentiate physiological stages:

-Initial lag phase where the cells adapt to the media, assimilate nutrients and grow in size, in this stage the population number remains constant.

-The exponential or logarithmic phase, now cells start to reproduce and population rapidly grows in number.

-Stationary state where the population reaches its limit established by the quantity of available nutrients. The own metabolism of the cells and other external factors affect the population and some cells die while some reproduce, the population remains constant.

Already since the begin of the first PEF studies it was demonstrated that cells in the exponential (or logarithmic) growth phase are more susceptible to the external electric field (Jacob 1981). This is believed to be a cause of damage in the membrane, a sort of scars, caused by the cell division. On the other hand, the highest resistance to the electric field is found in the first stage, where smaller concentrations and small cells are yet not affected by cell division scars or other damages. Algae cultures are cultivated with the intention to produce products. For this reason, it is reasonable to consider that microalgae will be treated at the stationary state, when its growth stage is maximum.

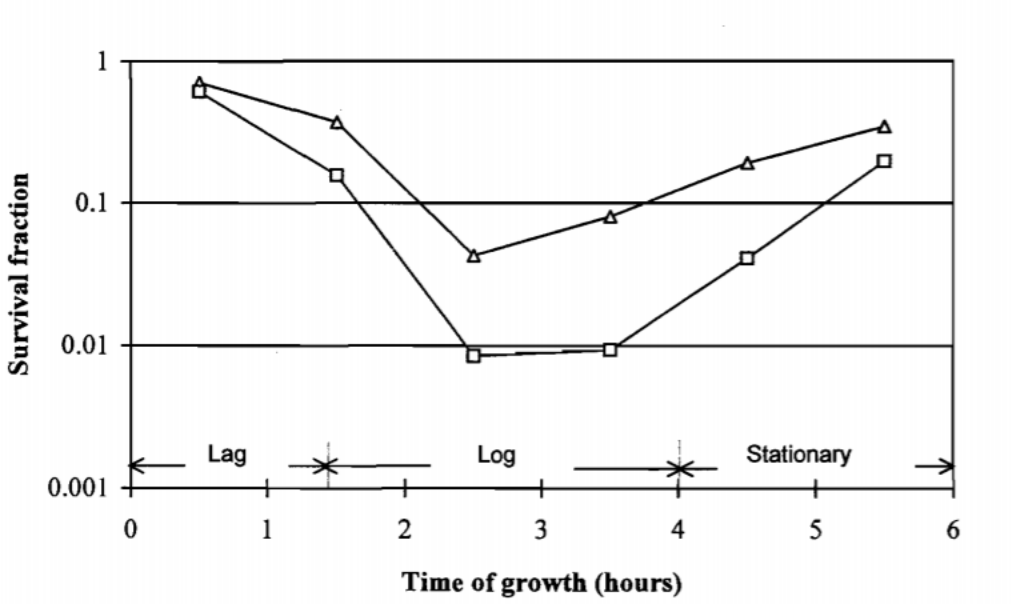

Figure 20. Effect of the growth stage in the survival rate by PEF treatment. The three stages of growing are marked as Lag (initial stage), Log (logarithmic growth stage) and Stationary (final static stage). Two different treatments with Δ 2 pulses and □ 4 pulses where performed (Pothakamury 1996).

5 Enhanced lipid extraction of algae with pulsed electric fields (PEF) for biodiesel

5.1 Global Energy Overview and biodiesel potential

It is well documented that the energy demand has dramatically increased in the last decades and that it will maintain the trend for the ones to come, it is reported that it will keep growing a 30% from 2017 to 2040 (World Energy Outlook, 2017). This situation goes hand by hand with the increase of greenhouse gases (GHGs) emissions such as Carbon dioxide (CO_2), Methane (CH_4) and Nitrous oxide(N_2O). Gas emissions from industry and transport are currently the main contributors to the GHGs emissions. These emissions come from different sources, the coil is the main source of energy in the industry while in transport is crude oils. The high dependence in fossil fuels is of major concern and there is a need for an economic, sustainable and environmentally friendly. Biofuels are a potential alternative to fossil fuels, as they are produced throw a relatively short biological process. There are a lot of biofuels but this paper is based in algae biodiesel. Biodiesel is a liquid fuel obtained from the lipids of plants (feedstock) or animals that can be directly used in the current engines without any modifications.

Figure 21 Change in primary energy demand (World Energy Outlook, 2017).

Biodiesels are usually divided in three major categories or generations:

First generation are those that come from terrestrial crops such as corn, soy bean, sugar cane, wheat etcetera. They are already commercialised in an industrial scale and are economically viable. However, there are two major problems related to their production. First, due to the increasing world population 7 billion and prevision of 9 billion by 2040 (by the united nations) there is need of arable land for food production. Thus, the competition for the arable land is a risk of price increase and social problems. Secondly, there is a huge controversy in the reduction of the GHGs emissions as many studies show deficiencies in the life cycle.

Second generation biodiesels feedstock does not compete with food production. They usually come from a wide range of biomass sources like forest residues and non-edible crops wastes.

Third generation biodiesels are algae. This are the latest phase of biofuels history and the most promising one as well as controversial. Algae do not compete with food feedstock for arable land, as it can grow in either open or closed tanks (ponds). It does not require “clean” water, as it can use waste water to feed from its nutrients. It does not produce CO_2 either, but instead, consumes it to grow. Algae also produce much more lipids than other feedstock and in much less time. Nevertheless, the counter part is that it needs a much higher investment and there are many more operating costs (Figure 23). While other crops have been cultivated for thousands of years with relatively simple technology, algae (at industrial scale production) need from a lot of cares and treatments. There is also a controversy of whether in its life cycle it produces net energy or not. There is a growing interest in the optimization of the algae production, not only for lipid (biodiesel) but also for other algae derived products such as drugs, fertilizers, pigments, nutritional supplements etcetera.

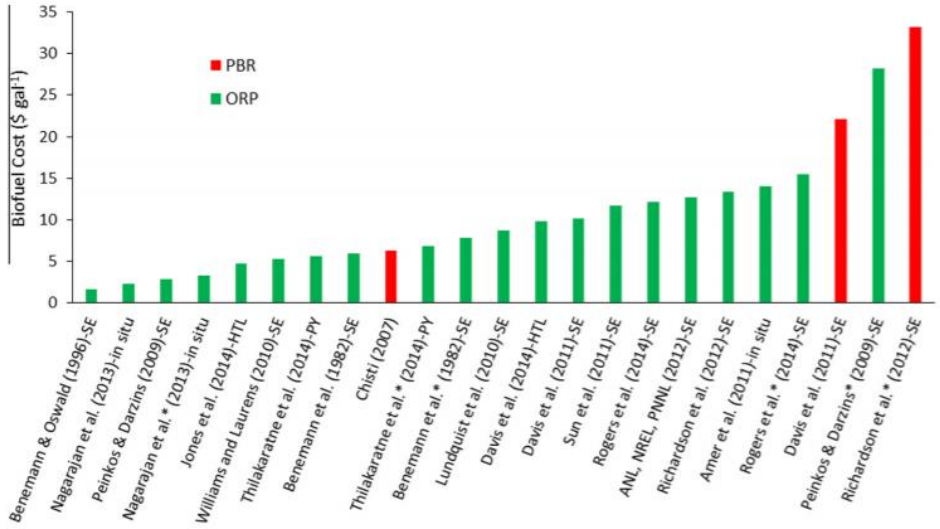

Figure 22 Techno economic studies of algae biofuels from different reports. Costs are reported in dollars and do consider an inflation rate to compare old reports with the more recent ones. The red colour stands for Photobioreactor (PBR) and the green for Open Race Pond (ORP) (Quinn 2014).

5.2 Algae lipid extraction

The lipids used for biofuel production are contained inside the algae cell walls. There are several methods to extract the lipids from within the cell. It is common to apply a pre-treatment to break the cell walls and then use solvent to extract the lipids however, it is not necessary. Whereas for terrestrial crops it is relatively easy to extract the lipids, algae are too small to use conventional techniques. There are many species of algae with a wide range of sizes from 2µm to 100µm and different properties. Therefore, each method has to be adjusted to each strain to operate at full potential.

One of the main problems of algae biofuel is its production costs (Tandon 2017). Although its many benefits, it must have a competitive price compared to that from fossil fuels to be feasible. One of the major contributors to the final cost of the process is the harvesting/lipid extraction phase which represent between 20% and 30% of the total costs (Mata 2010). The main reason for the high operating cost of this phase is the low density of algae populations, as they are diluted in water

medium with concentrations from 0,1 to 3 g/L (Halim 2012). Typically, before the lipid extraction the population goes throw a de-watering process to remove the water within the solution medium and enhance the lipid extraction performance. However, this process is energy and economically intensive (Mata 2010) a good solution could be the use of lipid extraction methods that can be applied directly in wet biomass (Halim 2011).

Typically, cell lysis methods can be divided in two categories: Mechanical and Non-Mechanical. Usually mechanical methods are highly effective but also require high energy input (Halim 2012). Many of these methods are derived from terrestrial feedstock and are not adjusted to algae. Others are only suitable for small laboratory analysis providing good results but inappropriate or impossible for scale up industry processing. It is usually considered that a proper extraction technique must be efficient, work directly with wet feedstock and be able to operate at large scale systems (National Algal Biofuels Technology Review, 2016). Another consideration to be economically viable is the extraction not only of algae lipids but other valuable products that may help to support the commercialization of biofuels (Davis 2014). Comparisons between techniques are difficult to perform as each study is done under different circumstances and many factors may affect the final lipid content extracted (Kim 2013).

5.2.1 Pulsed Electric Fields

PEF treatment is growing in popularity in food production and more recently in lipid extraction. It is based on the electroporation or electropermeabilitzation phenomenon widely used in genetic engineering for gene transfer. In biofuel production, it is often used as a pretreatment in combination with solvents. PEF pretreatment causes irreversible membrane breakdown (cell lysis) which promotes the release of intercellular substances. Thus, the solvent has full access to the cell products (Figure 24). It also has the advantage that it can work with wet biomass saving a lot of energy involved in de-watering, easy escalation to industrial size and it does not need a specific temperature nor adds additional impurities or damages the product (Goettel 2013). On the other hand, it is highly limited to the existence of air

bubbles which represent a risk to the safety and effectiveness of the process. Another major concern mostly overlooked by many studies as stated by Lam (2017) is the medium conductivity. This was considered one of the most influential factors in the effectiveness of the treatment, the lowest the better. Sea algae are specially related to this problem as the medium is more conductive. A solution to this problem could be washing algae with less conducting mediums (many laboratory experiments use ultrapure water Mili-Q) to increase the overall resistance, nevertheless further research is needed in the topic to provide more detailed analysis.

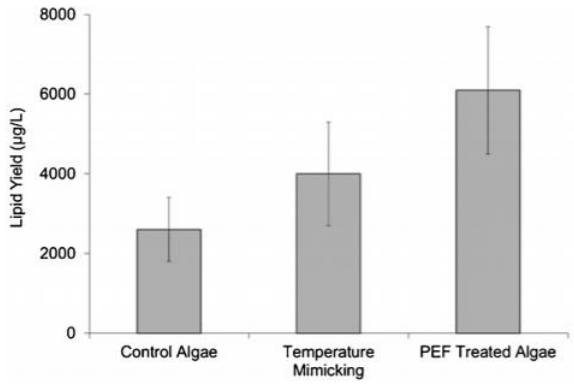

Figure 23. Extracted lipids from algae. Comparison of different pretreatments combined with ethyl acetate as solvent. Control Algae (fresh, without any pre-treatment), Temperature Mimicking (increased temperature equal to that produced for the PEF treatment), PEF Treated Algae (algae under pulsed electric fields) (Znibden 2013).

5.2.2 Solvent extraction

Solvent extraction (also known as liquid-liquid) is currently the most used methodology for algae lipid extraction as it is a simple and versatile technique. The combination of solvents extracts the mass from inside of the algae cells which permits the recovery of lipids. There is a wide range of solvent combinations (Figure 25) such as n-hexane/ethanol, DBU/ethanol, acetone/dichloromethane, dichloroethane/ethanol and more, however the most used mixtures are known as “Folch” (Folch 1957) and “Bligh and Dyer” (Blight and Dyer 1959). Both, named after their inventors, basically consist of a mixture of chloroform and methanol but differ

in the mixture ratios. There are also many variations of this methods adding different solvents to increase its performance.

While solvents are very effective they are considered as non appropriate for large scale operations due to the risks for the environment, health and safety. For this the quantity of used solvents is often reduced with pretreatments that implies solvent/physical extraction and require less solvent.

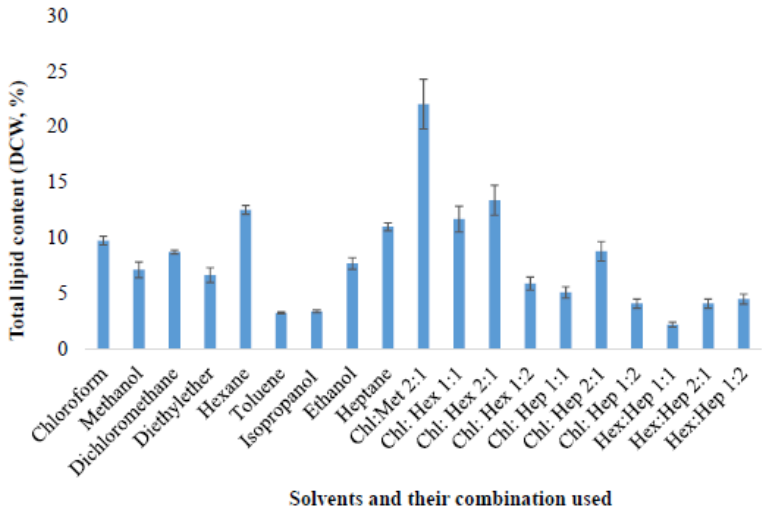

Figure 24 Lipid extraction percentage from dry biomass. Chl: chloroform, Hex:hexane, Hep:heptane, Met:methanol (Puri 2015).

5.2.3 High Pressure Homogenizer (HPH)

A mechanical process where piston pushes the microalgae throw a narrow orifice at high pressure (between 2kPsi to 45kPsi) which propels the algae at very high velocities. The shear forces created in the process break the cell walls allowing the lipid extraction (Samarasinghe 2012) One of the benefits is that the process is capable of scaling up.

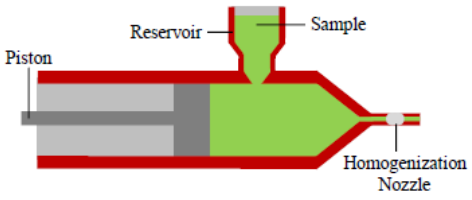

Figure 25 Adapted scheme of a High Pressure Homogenizer (HPH). (Samarasinghe 2012)

5.2.4 Bead Beating

A mechanical method where the biomass is spinning at high-speed. Several beads in the chamber cause destruction of the cell walls. Any kind of cells (or strains) can be disrupted in this process (Lee 1998). There are different variations, one of the most common one is the agitated beads. In this case the beads are also in movement, this increases the disruption effect. De-watering is not required what reduces the economic cost, however it does require a lot of energy and it is difficult to scale up (Roux 2017).

5.2.5 Microwave

A high frequency electric field (frequencies between 300MHz and 300GHz) applied to a polar or dielectric product causes movement of ions what secondly induces heat. This heating causes water evaporation. The energy can cause hydrogen bonds to break and changes in the membrane structure (Passos 2014).)This technology is popular due that it is simple as well as time efficient. The drawback is the maintenance cost of the process in large scale units (Muthu 2015).

5.2.6 Ultrasonic waves (Sonication)

Sonic waves applied to the product cause cell disruption by cavitation. It consists in the formation of micro-bubbles due to the rapid compression and decompressions cycles of the sonic waves. When the bubbles destabilize due to their own pressure they produce a strong implosion that produces a shock wave and disrupts the cell wall. The process can be scaled up and it is used in the chemical industry to homogenize solutions. It does not require much treatment time and it can be used with wet biomass, what highly reduces costs. As limitations, the release of free radicals due to the breakdown of water molecules may degrease the quality of the products and energy cost lost in heating and cooling to reduce it (Fanny 2012).

5.2.7 Supercritical CO_2 extraction

A gas (in most cases CO_2) is compressed and heated to convert it in a supercritical fluid where gaseous and liquid phases coexist. At this point the properties of both phases are combined resulting in the solubility of its liquid state and the diffusion of the gas. The process has a high extraction performance moreover, the extracted products are not contaminated or deteriorate and are easy to recover (separate) from the solvent. On the other hand, the drawbacks of this extraction process are the high energy input and difficulties for large scale use. It also requires for a pre-treatment to break the cell walls which involves more energy, costs and time (Mubarak 2015).

5.2.8 Osmotic pressure (or shock)

A Hypo-osmotic process occurs when the salt concentration in the interior is higher than that from the exterior. Hence, the medium fluid flows into the interior of the cell due to the osmotic pressure causing the cell walls to break. It is considered as a simple and cost-effective method however it requires long treatment times. Further research in the capability of large scale systems is still needed.

Method	Efficiency	Cost	Energy input	Remarks
Solvents	Moderate	High due to the solvents itself.	Energy intensive	Environment, health, fire hazards
High Pressure Homogenization	High	High	Energy intensive	Feasible to scale up
Supercritical CO_2	High	High cost	Energy intensive due to the phase transformation	Difficult to scale up
Bead Beating	Moderate	Cost-effective	Energy intensive	Difficult to scale up
Microwave	Very High	Initial investment and maintenance cost high	Energy intensive to process and to cool the product after	Feasible to scale up
Sonication	High	Initial investment and maintenance cost high	Energy intensive to process and to cool the product after	Poor product quality
Osmotic shock	Moderate	Low cost	Low energy input	Long treatment time
Pulsed Electric Fields	Very High	Initial cost and maintenance high, but operates at lower costs	Low energy input	Feasible to scale up

Table 1 Comparison of different lipid extraction methods. (National Algal Biofuels Technology Review, 2016) and (Muthu 2015)

Strain	Extraction method	Conditions	Lipid yield %	Efficiency %	Year
Botryococcus braunii	Solvent mixtures	Chloroform–methanol mixture (75% v/v of methanol)	19.2		2016
	Solvent mixtures	Petroleum ether–methanol (75% v/v of methanol)	18.90		
Chlorella sp. KR-1	Chemical	Potassium persulfate 2 mM, 90 °C		95	2016
	Chemical	Hydrogen peroxide 0.5%, 90 °C		80	
Chlorella pyrenoidosa	Solvent—mechanical	Ethanol, hydraulic press, 30 °C, 600 kPa	11.3	72	2016
Chlorella sp. KR-1	Solvent—UV light	UV–Fenton-like reaction, 0.5% H_2O_2, 16 W of UV light		87	2015
Tetraselmis sp.	Solvent—microwave	Hara and Radin method + MW, 65 °C at 5 min	8.19		2014
Nannochloropsis sp.	Solvent—microwave	Folch *et al.* method + MW, 65 °C at 5 min	8.47		
Chlorella vulgaris	Solvent—microwave	Soxhlet, chloroform: methanol (2:1), 8 (h). MW 15 min at 100 °C	22.68		2016
C. vulgaris	Enzymatic hydrolysis -solvent	2% (v/v) Celluclast 1.5 L and 1% (v/v) Novozyme 188, 72 (h). chloroform : methanol = 2:1 (v/v)	~10		2013
Scenedesmus sp.	Supercritical carbon dioxide (SC-CO_2)	53 °C, 500 bar, and 1.9 g min^{-1}	7.41		2014
C. vulgaris	Solvent—ultrasound	Bligh and Dyer method, ultrasonic bath 40 kHz, intensity of 29.7 W L^{-1}	52.5		2013
C. vulgaris	Electroporation	21 × 100 µs pulses, 4 kV, 10 Hz	22		2014

Table 2 Comparison between different extraction methods. Information extracted from sever studies (dates shown on "Year") (Arenas 2016).

5.3 Future Prospective

Algae biodiesel has been a controversial topic for many researchers. Although its many benefits and promising characteristics it still has many barriers to overcome. The major concerns of the technology are the high costs of the final product and the GHG emissions. Some LCA (Life Cycle Assessment) show a negative ratio of CO_2 emissions and a negative ratio of energy (more energy used to produce the biofuel that the one that this produces) (Lam 2012). However, both comparisons and conclusions are difficult to extract due to different production parameters and lack of standardization.

It is now assumed that the future of algae biodiesel will be closely related to the extraction of other valuable algae co-products and genetically modified strains. This way a major percentage of the algae components will be taken into account and

used to reduce costs and enhancing viability (Eppink 2017) and (Shurin 2016). Following this path and in order to use these products, it is important to use techniques that do not deteriorate valuable co-products. Pulsed Electric fields, between others, represent a feasible option for this purpose (Sean Lai 214).

It has also been stated, that to reduce the energy consumption further research in harvesting and lipid extraction techniques is required. Some consider that lipid extraction methods directly on wet biomass suppose the future on this field (Campbell 2011). De-watering is a highly energy intensive process and considered one of the bottlenecks of the production. PEF treatment permits lipid extraction directly in wet biomass hence, dramatically reducing the energy input and overall production costs. It is considered that although the energy requirements of the PEF treatment the saving from the dehydrating process are much higher.

It is obvious that for being a feasible technology it has to be implemented in an industrial scale. The fuel production market is PEF systems are already used in some large food process, for what its capability to scale up it is proved. Some companies recently developed specific PEF units for algae biodiesel (for example, but not only, diversified (Kempkes 2016) but further research is still needed to parametrise all factors involved in the process.

Improvements in treatment chambers designs are also required to industrialise the process. As explained in past chapters microalgae cultures have very low densities of cells, this requires from higher flow rates to maintain a good productivity. Parallel treatment chambers can be used to increase the flow without reducing the chamber resistivity. Yet improved chambers may come from either algae R+D or food production investigations.

Medium conductivity is another interesting field of improvement. Marine strains or other conductive medium may be an impediment for the utilisation an effectiveness of PEF. This is usually counter-effected with a washing treatment prior to the PEF. the use of low conductive mediums can dramatically reduce the energy consumption and enhance performance (Eing 2013).

6 Conclusions

Overall PEF treatment is a promising technology for lipid extraction at industrial level. Some corporations already developed industrial PEF units for microalgae treatment. However, further research is needed for the parametrization of energy efficiency and performance of this units in large production systems. Many factors have a strong influence in the results of the treatment consequently, the correct setting of each factor in essential to further improve the treatment results. Another point of interest is the genetic modification of algae strains to produce weaker membrananes and higher content of valuable products. This idea, whereas not unique of the PEF treatments, is in huge development at the moment as it permits major benefits of specific products or methodologies.

There is still a lot of research to do in the fields of electroporation, pulsed electric fields and algae biodiesel production. On the on hand, well understanding of the concept of electroporation could reveal new ways of optimization. Advances in PEF systems on the other hand, may not come directly from investigations on algae but, otherwise, from the field of food production. The increasing popularity of the method will be soon followed by more advances in large scale systems. However, research of the use of PEF treatment directly focused in algae is needed to completely understand its parameters and effects.

For its many critics algae biodiesel is a controversial topic, many improvements are still needed to become a competitive product. Problems related to the lipid extraction are currently one of the main areas of research thus, numerous technologies regarding this issue are being developed. Pulsed electric fields (PEF) is a complex technology that has the potential to optimise biodiesel production, however more improvements will be required to commercialise microalgae biodiesel.

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